L Number	Hits	Search Text	DB	Time stamp
1	790	helper same probe	USPAT; US-PGPUB; DERWENT	2003/06/27 11:54
2	714	(helper same probe) and 18S	USPAT; US-PGPUB; DERWENT	2003/06/27 11:54
3	140	helper near4 probe	USPAT; US-PGPUB; DERWENT	2003/06/27 11:54
4	14	(helper near4 probe) and 18S	USPAT; US-PGPUB; DERWENT	2003/06/27 11:59
5	117	"5030557"	USPAT; US-PGPUB; DERWENT	2003/06/27 11:56
6	2	(helper near4 probe) and 18S and interacting	USPAT; US-PGPUB; DERWENT	2003/06/27 11:59
7	2	(helper near4 probe) and 18S and ribofuranosyl	USPAT; US-PGPUB; DERWENT	2003/06/27 11:59

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=> d his

     (FILE 'HOME' ENTERED AT 08:31:00 ON 27 JUN 2003)

     FILE 'REGISTRY' ENTERED AT 08:31:13 ON 27 JUN 2003
L1            212 S CTATCAGCTTTAGACGGTAGGG/SQSN
L2              2 S L1 AND 23-100/SQL

     FILE 'CAPLUS' ENTERED AT 08:32:49 ON 27 JUN 2003
L3              1 S L2
                  S CTATCAGCTTT/SQSN

     FILE 'REGISTRY' ENTERED AT 08:34:34 ON 27 JUN 2003
L4          10736 S CTATCAGCTTT/SQSN

     FILE 'CAPLUS' ENTERED AT 08:34:58 ON 27 JUN 2003
L5           1194 S L4
L6              0 S L5 AND 18-100/SQL

     FILE 'REGISTRY' ENTERED AT 08:36:23 ON 27 JUN 2003
L7          10736 S CTATCAGCTTT/SQSN
L8             20 S L7 AND 18-100/SQL

     FILE 'CAPLUS' ENTERED AT 08:37:37 ON 27 JUN 2003
L9              5 S L8

     FILE 'REGISTRY' ENTERED AT 08:38:33 ON 27 JUN 2003
L10          1667 S AGACGGTAGGG/SQSN
L11             5 S L10 AND 18-100/SQL

     FILE 'CAPLUS' ENTERED AT 08:39:27 ON 27 JUN 2003
L12             2 S L11

=>
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```
=> s align?
L1          166900 ALIGN?

=> s parvum or crptosporidium
L2            9814 PARVUM OR CRPTOSPORIDIUM

=> s gondii or neurona or muris or gigantea or cruzi or capracanis or arieticansi
or tenella
L3           47809 GONDII OR NEURONA OR MURIS OR GIGANTEA OR CRUZI OR CAPRACANIS
                   OR ARIETICANSI OR TENELLA

=> s l2 and l3  and l1
L4              19 L2 AND L3 AND L1

=> dup reml 4
ENTER REMOVE, IDENTIFY, ONLY, OR (?):end

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5              13 DUP REM L4  (6 DUPLICATES REMOVED)

=> d ibib ab 1-13

L5   ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2003:356474  CAPLUS
DOCUMENT NUMBER:          138:362638
TITLE:                    Diagnosis and treatment of infectious diseases through
                          indel-differentiated proteins
INVENTOR(S):              Reiner, Neil E.; Tcherkassov, Artem; Nandan, Devki
PATENT ASSIGNEE(S):       The University of British Columbia, Can.
SOURCE:                   PCT Int. Appl., 64 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.          KIND  DATE          APPLICATION NO.  DATE
     ----------------    ----  --------      ---------------  --------
     WO 2003037926        A1   20030508      WO 2002-CA1689   20021101
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
             PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
             UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD,
             RU, TJ, TM
        RW:  GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
             CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
             NE, SN, TD, TG
PRIORITY APPLN. INFO.:                  CA 2001-2360987  A  20011101
                                        US 2002-349339P  P  20020122
                                        US 2002-349371P  P  20020122
                                        US 2002-393385P  P  20020705
AB   A compd. capable of specifically binding to pathogen EF-1.alpha. but not
     host EF-1.alpha., wherein the compd. binds to any part of an amino acid
     sequence having at least 70% sequence identity to amino acids 240-230 of
     SEQ ID NO:22 of EF-1.alpha. from Leishmania donovani.
REFERENCE COUNT:          10    THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5   ANSWER 2 OF 13     MEDLINE                              DUPLICATE 1
```

ACCESSION NUMBER: 2002312290 MEDLINE
DOCUMENT NUMBER: 22048751 PubMed ID: 12054017
TITLE: Molecular phylogeny and evolutionary relationships of Cryptosporidium parasites at the actin locus.
AUTHOR: Sulaiman Irshad M; Lal Altaf A; Xiao Lihua
CORPORATE SOURCE: Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, Georgia 30341, USA.
CONTRACT NUMBER: DW75937730-01-0
SOURCE: JOURNAL OF PARASITOLOGY, (2002 Apr) 88 (2) 388-94.
Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200206
ENTRY DATE: Entered STN: 20020611
Last Updated on STN: 20020628
Entered Medline: 20020627

AB To further validate previous observations in the taxonomy of Cryptosporidium parasites, the phylogenetic relationship was analyzed among various Cryptosporidium parasites at the actin locus. Nucleotide sequences of the actin gene were obtained from 9 putative Cryptosporidium species (C. **parvum**, C. andersoni, C. baileyi, C. felis, C. meleagridis, C. **muris**, C. saurophilum, C. serpentis, and C. wrairi) and various C. **parvum** genotypes. After multiple **alignment** of the obtained actin sequences, genetic distances were measured, and phylogenetic trees were constructed. Results of the analysis confirmed the presence of genetically distinct species within Cryptosporidium and various distinct genotypes within C. **parvum**. The phylogenetic tree constructed on the basis of the actin sequences was largely in agreement with previous results based on small subunit rRNA, 70-kDa heat shock protein, and Cryptosporidium oocyst wall protein genes. The Cryptosporidium species formed 2 major clades; isolates of C. andersoni, C. **muris**, and C. serpentis formed the first major group, whereas isolates of all other species, as well as various C. **parvum** genotypes, formed the second major group. Intragenotype variations were low or absent at this locus.

L5 ANSWER 3 OF 13 MEDLINE
ACCESSION NUMBER: 2001261987 MEDLINE
DOCUMENT NUMBER: 21215633 PubMed ID: 11318578
TITLE: Myosin diversity in Apicomplexa.
AUTHOR: Heintzelman M B; Schwartzman J D
CORPORATE SOURCE: Department of Anatomy, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: AI-34760 (NIAID)
SOURCE: JOURNAL OF PARASITOLOGY, (2001 Apr) 87 (2) 429-32. Ref: 16
Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF006627; GENBANK-AF066028; GENBANK-AF105117; GENBANK-AF105118; GENBANK-AF221131; GENBANK-AF222716; GENBANK-AF222717; GENBANK-AF273845; GENBANK-AF273846; GENBANK-AF273847; GENBANK-AF273848; GENBANK-AF273849; GENBANK-AF273850; GENBANK-AF273851; GENBANK-AF273852; GENBANK-AF273853; GENBANK-AF273854; GENBANK-AF273855; GENBANK-AF273856; GENBANK-AF273857; GENBANK-AF273858; GENBANK-AF273859; GENBANK-AF273860; GENBANK-AF273861;

GENBANK-AF273862; GENBANK-AF273863; GENBANK-AF273864;
GENBANK-AF273865; GENBANK-AF273866; GENBANK-AF273867;
GENBANK-AF273868; GENBANK-AF273869; GENBANK-AF273870;
GENBANK-AF273871; GENBANK-AF273872; GENBANK-AF273873;
GENBANK-AF273874; GENBANK-Z11718; GENBANK-AF006626;
GENBANK-P08799; GENBANK-P10568; SWISSPROT
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

AB A polymerase chain reaction (PCR) screen was used to examine the diversity of myosins in 7 Apicomplexan parasites: Toxoplasma **gondii**, Plasmodium falciparum, Neospora caninum, Eimeria **tenella**, Sarcocystis **muris**, Babesia bovis, and Cryptosporidium **parvum**. Using degenerate PCR primers compatible with the majority of known myosin classes, putative myosin sequences were obtained from all of these species. All of the sequences obtained showed greatest similarity to previously identified apicomplexan myosins, suggesting that the diversity of myosins in these parasites is limited. Myosin classes that are known to be widespread across the phylogenetic spectrum, e.g., the myosins I, II, and V, were not seen in the Apicomplexa. Thus, like the plants, the Apicomplexa may have evolved their own unique cohort of myosins that are responsible for the myosin-driven cellular functions observed in these parasites.

L5 ANSWER 4 OF 13 MEDLINE
ACCESSION NUMBER: 2001094625 MEDLINE
DOCUMENT NUMBER: 21030969 PubMed ID: 11191899
TITLE: Morphologic, host specificity, and genetic characterization of a European Cryptosporidium andersoni isolate.
AUTHOR: Sreter T; Egyed Z; Szell Z; Kovacs G; Nikolausz M; Marialigeti K; Varga I
CORPORATE SOURCE: Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary.
SOURCE: JOURNAL OF PARASITOLOGY, (2000 Dec) 86 (6) 1244-9.
Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125

AB This study was undertaken in order to characterize a Cryptosporidium **muris**-like parasite isolated from cattle in Hungary and to compare this strain with other Cryptosporidium species. To date, the large-type oocysts isolated from cattle were considered as C. **muris** described from several mammals. The size, form, and structure of the oocysts of the Hungarian strain were identical with those described by others from cattle. An apparent difference between the morphometric data of C. **muris**-like parasites isolated from cattle or other mammals was noted, which is similar in magnitude to the differences between Cryptosporidium meleagridis and Cryptosporidium felis or between Cryptosporidium serpentis and Cryptosporidium baileyi. The cross-transmission experiments confirmed the findings of others, as C. **muris**-like oocysts isolated from cattle fail to infect other mammals. The sequence of the variable region of small subunit (SSU) rRNA gene of the strain was 100% identical with that of the U.S. Cryptosporidium andersoni and C. andersoni-like isolates from cattle. The difference between the SSU rRNA sequence of bovine strains and C. **muris** is similar in magnitude to the differences between C. meleagridis and Cryptosporidium **parvum** anthroponotic genotype or

between Cryptosporidium wrairi and C. **parvum** zoonotic genotype. Our findings confirm that the Cryptosporidium species responsible for abomasal cryptosporidiosis and economic losses in the cattle industry should be considered a distinct species, C. andersoni Lindsay, Upton, Owens, Morgan, Mead, and Blagburn, 2000.

L5 ANSWER 5 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:520451 BIOSIS
DOCUMENT NUMBER: PREV199900520451
TITLE: Cryptosporidium is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences.
AUTHOR(S): Carreno, Ramon A.; Martin, Donald S.; Barta, John R. (1)
CORPORATE SOURCE: (1) Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, N1G 2W1 Canada
SOURCE: Parasitology Research, (Nov., 1999) Vol. 85, No. 11, pp. 899-904.
ISSN: 0932-0113.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The phylogenetic placement of gregarine parasites (Apicomplexa: Gregarinasina) within the Apicomplexa was derived by comparison of small-subunit ribosomal RNA gene sequences. Gregarine sequences were obtained from Gregarina niphandrodes Clopton, Percival, and Janovy, 1991, and Monocystis agilis Stein, 1848 (Eugregarinorida Leger 1900), as well as from Ophriocystis elektroscirrha McLaughlin and Myers, 1970 (Neogregarinorida Grasse 1953). The sequences were **aligned** with several other gregarine and apicomplexan sequences from GenBank and the resulting data matrix analyzed by parsimony and maximum-likelihood methods. The gregarines form a monophyletic clade that is a sister group to Cryptosporidium spp. The gregarine/Cryptosporidium clade is separate from the other major apicomplexan clade containing the coccidia, adeleids, piroplasms, and haemosporinids. The trees indicate that the genus Cryptosporidium has a closer phylogenetic affinity with the gregarines than with the coccidia. These results do not support the present classification of the Cryptosporidiidae in the suborder Eimerioirina Leger, 1911.

L5 ANSWER 6 OF 13 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999313018 MEDLINE
DOCUMENT NUMBER: 99313018 PubMed ID: 10386447
TITLE: Phylogenetic analysis of Cryptosporidium isolates from captive reptiles using 18S rDNA sequence data and random amplified polymorphic DNA analysis.
AUTHOR: Morgan U M; Xiao L; Fayer R; Graczyk T K; Lal A A; Deplazes P; Thompson R C
CORPORATE SOURCE: World Health Organisation Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, Murdoch University, Western Australia, Australia.
SOURCE: JOURNAL OF PARASITOLOGY, (1999 Jun) 85 (3) 525-30.
Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF108863; GENBANK-AF108864; GENBANK-AF108865; GENBANK-AF108866
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990714
Last Updated on STN: 19990714
Entered Medline: 19990701

AB Sequence **alignment** of a polymerase chain reaction-amplified 713-base pair region of the Cryptosporidium 18S rDNA gene was carried out on 15 captive reptile isolates from different geographic locations and compared to both Cryptosporidium **parvum** and Cryptosporidium **muris** isolates. Random amplified polymorphic DNA (RAPD) analysis was also performed on a smaller number of these samples. The data generated by both techniques were significantly correlated ($P < 0.002$), providing additional evidence to support the clonal population structure hypothesis for Cryptosporidium. Phylogenetic analysis of both 18S sequence information and RAPD analysis grouped the majority of reptile isolates together into 1 main group attributed to Cryptosporidium serpentis, which was genetically distinct but closely related to C. **muris**. A second genotype exhibited by 1 reptile isolate (S6) appeared to be intermediate between C. serpentis and C. **muris** but grouped most closely with C. **muris**, as it exhibited 99.15% similarity with C. **muris** and only 97.13% similarity with C. serpentis. The third genotype identified in 2 reptile isolates was a previously characterized 'mouse' genotype that grouped closely with bovine and human C. **parvum** isolates.

L5 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:103927 CAPLUS
DOCUMENT NUMBER: 130:347910
TITLE: Sequence and PCR-RFLP analysis of the internal transcribed spacers of the rDNA repeat unit in isolates of Cryptosporidium from different hosts
AUTHOR(S): Morgan, U. M.; Deplazes, P.; Forbes, D. A.; Spano, F.; Hertzberg, H.; Sargent, K. D.; Elliot, A.; Thompson, R. C. A.
CORPORATE SOURCE: World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia
SOURCE: Parasitology (1999), 118(1), 49-58
CODEN: PARAAE; ISSN: 0031-1820
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Cryptosporidium ITS1, 5.cntdot.8S and ITS2 rDNA regions from a no. of Cryptosporidium isolates from different hosts and geog. areas were cloned and sequenced in order to investigate the extent of sequence heterogeneity between human and cattle-derived isolates from different geog. locations and also between isolates of Cryptosporidium from different hosts such as cats, pigs, mice and a koala. Calf-derived isolates from different continents were virtually identical as were human-derived isolates from the UK and Australia. Genetic differences between Cryptosporidium isolates were extensive and were in fact greater than the level of nucleotide divergence between Toxoplasma **gondii** and Neospora caninum rDNA sequences. Based on the sequence information derived from this study, PCR-RFLP of the ITS1 region was undertaken in order to directly amplify and genotype Cryptosporidium isolates from different hosts. This PCR-RFLP approach can now be used for mol. epidemiol. studies, circumventing the need for costly sequencing and allowing a wider range of genetically different isolates to be examd.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 13 MEDLINE
ACCESSION NUMBER: 1998360211 MEDLINE
DOCUMENT NUMBER: 98360211 PubMed ID: 9695098
TITLE: Molecular characterization of Cryptosporidium from various hosts.
AUTHOR: Morgan U M; Sargent K D; Deplazes P; Forbes D A; Spano F;

Hertzberg H; Elliot A; Thompson R C
CORPORATE SOURCE: World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, Murdoch University, Australia.. morgan@numbat.murdoch.edu.au
SOURCE: PARASITOLOGY, (1998 Jul) 117 (Pt 1) 31-7.
Journal code: 0401121. ISSN: 0031-1820.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF099666; GENBANK-AF099667; GENBANK-AF099668; GENBANK-AF099669; GENBANK-AF102766; GENBANK-AF102767; GENBANK-AF102768
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980903
Last Updated on STN: 20000303
Entered Medline: 19980827

AB A 298 bp region of the Cryptosporidium **parvum** 18S rDNA and a 390 bp region of the acetyl-CoA synthetase gene were sequenced for a range of human and animal isolates of Cryptosporidium from different geographical areas. A distinct genotype is common to isolates from cattle, sheep and goats and also an alpaca from Peru and is referred to here as the 'calf'-derived Cryptosporidium genotype. Another genotype of 'human'-derived isolates also appears to be conserved amongst human isolates although humans are also susceptible to infection with the 'calf' Cryptosporidium genotype. Mice and pigs carry genetically distinct genotypes of Cryptosporidium. Three snake isolates were also analysed, 2 of which exhibited C. **muris** genotypes and the third snake isolate carried a distinct 'mouse' genotype.

L5 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:75624 BIOSIS
DOCUMENT NUMBER: PREV199800075624
TITLE: Investigating phylogenetic relationships within the Apicomplexa using sequence data: The search for homology.
AUTHOR(S): Barta, John R. (1)
CORPORATE SOURCE: (1) Dep. Pathobiol., Univ. Guelph, Guelph, ON N1G 2W1 Canada
SOURCE: Methods (Orlando), (Oct., 1997) Vol. 13, No. 2, pp. 81-88.
ISSN: 1046-2023.
DOCUMENT TYPE: General Review
LANGUAGE: English

AB Whether stated explicitly or not, all molecular studies that seek to infer "homologies" among sequences or that attempt to determine the "relatedness" of taxa based on sequence comparisons are evolutionary studies. The generation of a reliable evolutionary hypothesis based on molecular sequences is dependent almost exclusively on the ability to **align** sequences such that bases or amino acids in the same position of two sequences are positionally homologous (i.e., they share the same position in the gene under study). The selection of suitable gene targets (commonly 18S small subunit rRNA gene sequences in the Apicomplexa) and appropriate ingroup and outgroup taxa will affect the ability to **align** sequences unambiguously. Mathematically derived **alignments** based on local sequence similarity have been shown to be less reliable than **alignments** based on conserved secondary structures coupled with an analysis of compensatory base changes. Use of staggered sequence **alignments** through hypervariable regions of 18S small subunit rRNA gene sequences in which subsets of taxa are **aligned** independently may permit inclusion of more of the primary sequences with an associated increase in information content in the data set. The use of these highly variable regions is critical for determining the branching order of closely related terminal taxa in the phylum Apicomplexa.

L5 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:534945 BIOSIS
DOCUMENT NUMBER: PREV199598549245
TITLE: Effects of sequence **alignment** on the phylogeny of Sarcocystis deduced from 18S rDNA sequences.
AUTHOR(S): Ellis, John (1); Morrison, David
CORPORATE SOURCE: (1) Dep. Cell Mol. Biol., Univ. Technol. Sydney, Gore Hill, NSW Australia
SOURCE: Parasitology Research, (1995) Vol. 81, No. 8, pp. 696-699. ISSN: 0932-0113.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The family Sarcocystidae contains a wide variety of parasitic protozoa, some of which are important pathogens of livestock and humans. The taxonomic relationships between two of the genera in this family (Toxoplasma and Sarcocystis) have been debated for a number of years and remain controversial. Recent studies, from comparisons of 18S rDNA-sequence data, have suggested that Sarcocystis is paraphyletic, although a hypothesis supporting monophyly of Sarcocystis could not be rejected. The present study shows that the phylogenetically informative nucleotide positions within the 18S rDNA are primarily located in the regions that make up the helices in the secondary structure of the 18S rRNA. A phylogenetic analysis of 18S rDNA-sequence data **aligned** by secondary structure constraints, or a subset of the data corresponding to all nucleotides found in the helices, provide unambiguous evidence supporting monophyly of Sarcocystis.

L5 ANSWER 11 OF 13 MEDLINE
ACCESSION NUMBER: 95348882 MEDLINE
DOCUMENT NUMBER: 95348882 PubMed ID: 7623193
TITLE: Sequence analysis and comparison of ribosomal DNA from bovine Neospora to similar coccidial parasites.
AUTHOR: Marsh A E; Barr B C; Sverlow K; Ho M; Dubey J P; Conrad P A
CORPORATE SOURCE: Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis 95616, USA.
SOURCE: JOURNAL OF PARASITOLOGY, (1995 Aug) 81 (4) 530-5. Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U17345; GENBANK-U17346; GENBANK-U17347; GENBANK-U17349; GENBANK-U25043; GENBANK-U25044
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950911
Last Updated on STN: 19950911
Entered Medline: 19950829
AB The nuclear small subunit ribosomal RNA (nss-rRNA) gene sequence of Neospora spp. isolated from cattle was analyzed and compared to the sequences from several closely related cyst-forming coccidial parasites. Double-stranded DNA sequencing of 5 bovine Neospora spp. isolates (BPA1-4), 2 Neospora caninum isolates (NC-1 and NC-3), and 3 Toxoplasma **gondii** isolates (RH, GT-1, CT-1) were performed and compared to each other, as well as to other sequences available in GenBank for the NC-1 isolate, Sarcocystis **muris**, and Cryptosporidium **parvum**. There were no nucleotide differences detected between the Neospora spp. isolates from cattle and dogs. Four nucleotide differences were consistently detected when sequences of Neospora spp. isolates were compared to those of the T. **gondii** isolates. These results indicate that Neospora spp. and T. **gondii** are closely related, but distinct, species.

L5 ANSWER 12 OF 13 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 95097110 MEDLINE
DOCUMENT NUMBER: 95097110 PubMed ID: 7799170
TITLE: Phylogenetic relationship of Sarcocystis **neurona** to other members of the family Sarcocystidae based on small subunit ribosomal RNA gene sequence.
AUTHOR: Fenger C K; Granstrom D E; Langemeier J L; Gajadhar A; Cothran G; Tramontin R R; Stamper S; Dubey J P
CORPORATE SOURCE: Department of Veterinary Sciences, University of Kentucky, Lexington 40546.
SOURCE: JOURNAL OF PARASITOLOGY, (1994 Dec) 80 (6) 966-75. Journal code: 7803124. ISSN: 0022-3395.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U07812
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19950215
Entered Medline: 19950125

AB Sarcocystis **neurona** is a coccidial parasite that causes a neurologic disease of horses in North and South America. The natural host species are not known and classification is based on ultrastructural analysis. The small subunit ribosomal RNA (SSURNA) gene of S. **neurona** was amplified using polymerase chain reaction techniques and sequenced by Sanger sequencing reactions. The sequence was compared with partial sequences of S. **muris**, S. **gigantea**, S. **tenella**, S. **cruzi**, S. arieticanis, S. **capracanis**, Toxoplasma **gondii**, Eimeria **tenella**, and Cryptosporidium **parvum**. **Alignments** of available sites for all 10 species and **alignments** of the entire SSURNA sequence of S. **neurona**, S. **muris**, S. **cruzi**, T. **gondii**, and C. **parvum** were performed. **Alignments** were analyzed using maximum parsimony and maximum likelihood methods to determine relative phylogeny of these organisms. These analyses confirmed placement of S. **neurona** in the genus Sarcocystis and suggested a close relationship to S. **muris**, S. **gigantea**, and T. **gondii**. Molecular phylogeny suggests that Sarcocystis spp., which utilize the dog (Canis familiaris) as the definitive host, evolved from a common ancestor, whereas those species (including T. **gondii**) that utilize the cat (Felis domesticus) as the definitive host evolved from another common ancestor. This suggests a possible definitive host for S. **neurona**.

L5 ANSWER 13 OF 13 MEDLINE
ACCESSION NUMBER: 92292035 MEDLINE
DOCUMENT NUMBER: 92292035 PubMed ID: 1818196
TITLE: Identification and isolation of Cryptosporidium **parvum** genes encoding microtubule and microfilament proteins.
AUTHOR: Nelson R G; Kim K; Gooze L; Petersen C; Gut J
CORPORATE SOURCE: Parasitology Laboratory, Department of Medicine, San Francisco General Hospital, University of California 94143.
CONTRACT NUMBER: AI07988 (NIAID)
AI29882 (NIAID)
AI29886 (NIAID)
+
SOURCE: JOURNAL OF PROTOZOOLOGY, (1991 Nov-Dec) 38 (6) 52S-55S. Journal code: 2985197R. ISSN: 0022-3921.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199207

ENTRY DATE: Entered STN: 19920724
Last Updated on STN: 19970203
Entered Medline: 19920714

AB Microtubules and microfilaments are highly conserved cytoskeletal polymers hypothesized to play essential biomechanical roles in the unusual gliding motility of Apicomplexan zoites and in their invasion of, and development within, host epithelial cells. We have identified and isolated Cryptosporidium **parvum** genes encoding the microtubule proteins alpha- and beta-tubulin and the microfilament protein actin by screening a lambda gt11 C. **parvum** genomic DNA library with degenerate oligonucleotide and heterologous cDNA hybridization probes respectively. The alpha- and beta-tubulin genes have been partially sequenced and the deduced peptide sequences show greatest homology with the tubulins of the related parasites, T. **gondii** and P. falciparum. The complete nucleic acid sequence of the actin gene predicts a 376 amino acid, 42 kDa protein having 85% sequence identity with the P. falciparum actin I and the human gamma-actin proteins. Each of these cytoskeletal protein genes was demonstrated to be of cryptosporidial origin by Southern analyses of C. **parvum** chromosomes fractionated by pulsed field gel electrophoresis; the cloned alpha- and beta-tubulin genes hybridized with chromosomes of ca. 1,200 and 1,500 kb respectively and the cloned actin gene also hybridized with a 1,200 kb chromosome.

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L7 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2001669065 MEDLINE
DOCUMENT NUMBER: 21571769 PubMed ID: 11714523
TITLE: Real-time PCR for the detection of Cryptosporidium **parvum**.
AUTHOR: Higgins J A; Fayer R; Trout J M; Xiao L; Lal A A; Kerby S; Jenkins M C
CORPORATE SOURCE: USDA-ARS, Rm. 202, Bldg. 173, 10300 Baltimore Blvd., Beltsville, MD 20705, USA.. jhiggins@anri.barc.usda.gov
SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (2001 Dec) 47 (3) 323-37.
Journal code: 8306883. ISSN: 0167-7012.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20011121
Last Updated on STN: 20020308
Entered Medline: 20020307

AB Real time, TaqMan PCR assays were developed for the Cp11 and **18S** rRNA genes of the protozoan parasite Cryptosporidium **parvum**. The TaqMan probes were **specific** for the **genus** Cryptosporidium, but could not hybridize exclusively with human-infectious C. **parvum** species and genotypes. In conjunction with development of the TaqMan assays, two commercial kits, the Mo Bio UltraClean Soil DNA kit, and the Qiagen QIAamp DNA Stool kit, were evaluated for DNA extraction from calf diarrhea and manure, and potassium dichromate and formalin preserved human feces. Real-time quantitation was achieved with the diarrhea samples, but nested PCR was necessary to detect C. **parvum** DNA in manure and human feces. Ileal tissues were obtained from calves at 3, 7, and 14 days post-infection, and DNA extracted and assayed. Nested PCR detected C. **parvum** DNA in the 7-day post-infection sample, but neither of the other time point samples were positive. These results indicate that real-time quantitation of C. **parvum** DNA, extracted using the commercial kits, is feasible on diarrheic feces, with large numbers of oocysts and small concentrations of PCR inhibitor(s). For samples with few oocysts and high concentrations of PCR inhibitor(s), such as manure, nested PCR is necessary for detection.

L7 ANSWER 2 OF 3 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000429837 MEDLINE
DOCUMENT NUMBER: 20419416 PubMed ID: 10966225
TITLE: Detection and speciation of Cryptosporidium spp. in environmental water samples by immunomagnetic separation, PCR and endonuclease restriction.
AUTHOR: Lowery C J; Moore J E; Millar B C; Burke D P; McCorry K A; Crothers E; Dooley J S
CORPORATE SOURCE: Department of Applied Biological and Chemical Sciences, University of Ulster, Coleraine, Northern Ireland.
SOURCE: JOURNAL OF MEDICAL MICROBIOLOGY, (2000 Sep) 49 (9) 779-85.
Journal code: 0224131. ISSN: 0022-2615.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000922
Last Updated on STN: 20000922
Entered Medline: 20000908

AB Current methods for the detection of Cryptosporidium oocysts in water samples are both time-consuming and subject to variation in sensitivity. A **genus-specific** PCR assay was designed for the

specific amplification of a 552-bp region of the **18S** rRNA gene. Postamplification endonuclease restriction generated unique digest patterns that enabled differentiation between the three species, C. muris, C. baileyi and C. **parvum**, the major human pathogen. Theoretical restriction profiles for other Cryptosporidium species were also predicted. The assay routinely detected 10 oocysts in 10-ml purified oocyst preparations, but sensitivity was found to be 10(3)-10(4) -fold lower in environmental water samples. The use of Chelex resin and an immunomagnetic separation procedure overcame this inhibition. This provided detection levels of 10(1)-10(3) oocysts, depending on water turbidity. Rapid and sensitive pathogen detection methods are essential for the water industry. The results of this study demonstrate that PCR has the potential to improve current detection capabilities greatly by differentiating the major human pathogens from non-pathogenic species. This will greatly facilitate a closer examination of the epidemiology of this important pathogen.

L7 ANSWER 3 OF 3 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 97133950 MEDLINE
DOCUMENT NUMBER: 97133950 PubMed ID: 8979344
TITLE: Comparison of primers and optimization of PCR conditions for detection of Cryptosporidium **parvum** and Giardia lamblia in water.
AUTHOR: Rochelle P A; De Leon R; Stewart M H; Wolfe R L
CORPORATE SOURCE: Water Quality Laboratory, Metropolitan Water District of Southern California, La Verne 91750-3399, USA.. prochelle@mwd.dst.ca.us
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Jan) 63 (1) 106-14.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970327
Last Updated on STN: 19970327
Entered Medline: 19970318

AB Eight pairs of published PCR primers were evaluated for the specific detection of Cryptosporidium **parvum** and Giardia lamblia in water. Detection sensitivities ranged from 1 to 10 oocysts or cysts for purified preparations and 5 to 50 oocysts or cysts for seeded environmental water samples. Maximum sensitivity was achieved with two successive rounds of amplification and hybridization, with oligonucleotide probes detected by chemiluminescence. Primer annealing temperatures and MgCl2 concentrations were optimized, and the specificities of the primer pairs were determined with closely related species. Some of the primers were species **specific**, while others were only **genus specific**. Multiplex PCR for the simultaneous detection of Cryptosporidium and Giardia was demonstrated with primers amplifying 256- and 163-bp products from the **18S** rRNA gene of Cryptosporidium and the heat shock protein gene of Giardia, respectively. The results demonstrate the potential utility of PCR for the detection of pathogenic protozoa in water but emphasize the necessity of continued development.

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     (FILE 'HOME' ENTERED AT 10:39:27 ON 26 JUN 2003)

      FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 10:42:36 ON 26 JUN 2003
L1        166900 S ALIGN?
L2          9814 S PARVUM OR CRPTOSPORIDIUM
L3         47809 S GONDII OR NEURONA OR MURIS OR GIGANTEA OR CRUZI OR CAPRACANIS
L4            19 S L2 AND L3   AND L1
L5            13 DUP REM L4 (6 DUPLICATES REMOVED)
L6             9 S 18S AND L2 AND (GENUS (5A) SPECIFIC)
L7             3 DUP REM L6 (6 DUPLICATES REMOVED)

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L Number	Hits	Search Text	DB	Time stamp
1	3375	cryposporidium or parvum	USPAT; US-PGPUB; DERWENT	2003/06/26 09:08
2	2905056	align$9 or compar$9	USPAT; US-PGPUB; DERWENT	2003/06/26 09:34
3	91	(cryposporidium or parvum) same (align$9 or compar$9)	USPAT; US-PGPUB; DERWENT	2003/06/26 09:52
4	140	parvum same (gondii or neurona or muris or gigantea or cruzi or capracans or tenella or arieticansi or tenella)	USPAT; US-PGPUB; DERWENT	2003/06/26 09:53
5	14	(parvum same (gondii or neurona or muris or gigantea or cruzi or capracans or tenella or arieticansi or tenella)) same (align$9 or compar$9)	USPAT; US-PGPUB; DERWENT	2003/06/26 09:54
6	93	(parvum same (gondii or neurona or muris or gigantea or cruzi or capracans or tenella or arieticansi or tenella)) and (PCR or probe or oligo$ or primer$)	USPAT; US-PGPUB; DERWENT	2003/06/26 09:55
7	79	((parvum same (gondii or neurona or muris or gigantea or cruzi or capracans or tenella or arieticansi or tenella)) and (PCR or probe or oligo$ or primer$)) not ((cryposporidium or parvum) same (align$9 or compar$9))	USPAT; US-PGPUB; DERWENT	2003/06/26 09:56
8	78	(((parvum same (gondii or neurona or muris or gigantea or cruzi or capracans or tenella or arieticansi or tenella)) and (PCR or probe or oligo$ or primer$)) not ((cryposporidium or parvum) same (align$9 or compar$9))) and (align$9 or compar$9)	USPAT; US-PGPUB; DERWENT	2003/06/26 09:56